

VITAMINS AND OTHER NUTRIENTS

Apparent Nitrogen Digestibility Data: AACC-ASTM Collaborative Study

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Apparent nitrogen digestibility data were obtained from 4 laboratories for 6 protein sources and 2 diet levels, 6 and 10% protein, after a 2-day adaptation period during the AACC-ASTM protein efficiency ratio (PER) and net protein ratio (NPR) collaborative studies. For 5 protein sources fed as 10% of the diet, the interlaboratory variation as measured by coefficient of variation (CV) values was low (1.5–3.5%), indicating high precision of the method. Wheat flour (6% protein diet) had the highest variation and, therefore, the lowest precision (CV of 7.10%). The interlaboratory variation (CV value) for 3 of the 4 laboratories was considerably lower, less than half that for the 4 laboratories. An analysis of variance of apparent nitrogen digestibility data indicated significant ($P < 0.05$) effects for the 4-laboratory group due to laboratories and protein diets at both 10 and 6% protein levels, and for the 3-laboratory group at the 10% protein level. The 3-laboratory ANOVA for the 6% diets indicated a significant effect ($P < 0.05$) due to diet only.

The use of dietary protein by the body for growth and maintenance is dependent not only on the presence and relative amounts of the essential amino acids, but also on digestibility of the protein, and utilization of the released amino acids (1). At the turn of the century, Atwater and colleagues (2) determined and used coefficients of digestibility to calculate energy values of food groups (3–5). Recently, the rationale for estimating protein nutritional quality of foods from amino acid composition data with and without corrections for digestibility data was discussed by Pellet and Young (6). The use of this approach to predict protein nutritional value for adults was reviewed by Bodwell (7). The effects of the variation of protein digestibility were reviewed by Hopkins (5), and Ritchey and Taper reported on the estimation of protein digestibility for humans from rat assays (8). It was the consensus at the Airlie Conference on "Protein Quality in Humans: Assessment and *In Vitro* Estimation," March 23–26, 1980, that corrections to allow for differences in nitrogen digestibility and/or essential amino acid bioavailability be included in amino acid scoring procedures that are used to estimate protein nutritive quality of foods (9).

Apparent nitrogen digestibility is a valuable parameter used in estimating protein nutritional quality of a food (5). Apparent nitrogen digestibility can be obtained during determination of protein efficiency ratio (PER), net protein ratio (NPR),

or other rat feeding tests, with only a small increase in work. There has been considerable interest in obtaining information on the interlaboratory variability of apparent nitrogen digestibility. Accordingly, this was included in the AACC-ASTM collaborative study on PER and NPR bioassays (10–12). Four of the collaborators agreed to participate in this aspect of the study.

Experimental

Apparent nitrogen digestibility data were collected by 3 laboratories by using a modified Mitchell procedure (13) and equations defined in other publications (6, 14–16) during the AACC-ASTM PER and NPR collaborative studies on only those rats subjected to a 2-day adaptation period before being fed the PER-NPR test diets (10–12). A fourth laboratory (Laboratory 3) collected apparent nitrogen digestibility data during a separate digestibility study using the same experimental procedures. Five test diets containing 10% protein (ANRC casein, lactalbumin, lyophilized lean beef, textured vegetable protein, and peanut flour) and 3 test diets containing 6% protein (ANRC casein, wheat flour, and textured vegetable protein) were fed by all collaborators during the digestibility determinations. There were 10 rats per diet. Filter paper, other similar absorbent paper, or absorbent boards, were used in the bottom of the cages to absorb urine and minimize possible urine contamination of the feces. The feces from each rat on a diet was collected on days 10 through 14 by a feeding-collection time schedule and carefully separated from any spilled food. The feces from the 10 rats in a diet group were composited and dried overnight in an oven at about 100°C. Each composited dried feces sample was equilibrated to room temperature and humidity, and was then ground, mixed thoroughly, and analyzed for nitrogen by AOAC method 2.057 and for moisture by AOAC method 24.003 (17). Feed intake was determined by monitoring uneaten food. Scattered food was carefully collected and all extraneous matter was separated before the food was weighed and analyzed for moisture and nitrogen (17). The weight of uneaten food was deducted from the weight of food offered to the animals to obtain the weight of food consumed. Apparent nitrogen digestibility and apparent diet digestibility were calculated on a moisture-free basis, using the following equations (5, 6, 14–16):

Apparent diet digestibility

$$= \frac{\text{feed intake} - \text{fecal weight}}{\text{feed intake}} \times 100$$

Apparent nitrogen digestibility

$$= \frac{\text{nitrogen intake} - \text{fecal nitrogen}}{\text{nitrogen intake}} \times 100$$

Results and Discussion

The percent apparent nitrogen digestibility for each protein diet from each laboratory, the mean of the values from 3 (Labs 3, 5, and 7) and from 4 laboratories (Labs 2, 3, 5, and

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Table 1. Percent apparent nitrogen digestibility data^a for 6 protein sources

Protein source	Laboratory				4 Laboratories ^b			3 Laboratories ^d		
	2	3	5	7	Mean	SD	CV, ^c %	Mean	SD	CV, ^c %
10% Protein Diets										
Casein	94.4	91.7	92.5	91.4	92.5	1.35	1.46	91.9	0.57	0.62
Beef	94.4	91.7	92.2	91.4	92.4	1.36	1.47	91.8	0.40	0.44
Lactalbumin	95.5	92.0	91.9	92.3	92.9	1.72	1.86	92.1	0.21	0.23
Peanut flour	94.5	89.3	89.8	87.4	90.2	3.02	3.34	88.8	1.27	1.42
Text. veg. protein	92.1	86.1	87.6	85.1	87.7	3.09	3.53	86.3	1.26	1.46
6% Protein Diets										
Casein	93.0	89.1	89.5	88.1	89.9	2.13	2.37	88.9	0.72	0.81
Wheat flour	92.5	83.8	81.4	78.7	84.1	5.97	7.10	81.3	2.55	3.14
Text. veg. protein	91.2	86.5	85.4	83.8	86.7	3.18	3.67	85.2	1.36	1.59

^aCalculated on a moisture-free basis.^bValues calculated for data from Laboratories 2, 3, 5, and 7.^cCoefficient of variation = SD/mean × 100.^dValues for data from Laboratories 3, 5, and 7.

7), the standard deviation (SD) of those means, and the coefficients of variation (CV) are given in Table 1. The apparent nitrogen digestibility values for each diet were in close agreement for 3 laboratories (Table 1). The fourth laboratory reported consistently higher results. The interlaboratory variation as measured by CV values is low, indicating high precision of the method on all 5 protein sources fed as 10% of the diet from either the 3-laboratory or 4-laboratory data. However, the 3-laboratory data exhibited much less variation, indicating increased precision. Wheat flour (6% diet) had the highest variation, and therefore the lowest precision. The intralaboratory variation could not be determined because the feces were composited for the 10 rats in each trial. Data from an analysis of variance (ANOVA) calculated over protein sources and 4 laboratories are shown in Table 2. The interlaboratory variability is low for the nitrogen digestibility of the five 10% protein diets. Although doubled for the three 6% protein diets, the level of variability was still acceptable (Table 2).

In conclusion, apparent nitrogen digestibility data indicated high precision among the 3 laboratories (Labs 3, 5, and 7) and acceptable precision for 4 laboratories (Labs 2, 3, 5, and 7), as measured by the coefficient of variation. However, an ANOVA indicated significant ($P < 0.05$) effects due to laboratories and diets at the 10% protein level for the 3- and 4-laboratory groups and at the 6% protein level for the 4-laboratory group. An ANOVA for the 3 laboratories showed a significant effect ($P < 0.05$) due to diet only for the 6% diet level data.

Table 2. Interlaboratory variability for digestibility data over laboratories and protein sources

Source	Mean, ^a %	Variance ^b	SD	CV, ^b %
Over 4 Laboratories (2, 3, 5, and 7)				
10% Protein diets (5):				
Nitrogen	91.2	5.03	2.24	2.46
Diet	90.9	4.02	2.01	2.21
6% Protein diets (3):				
Nitrogen	86.9	16.8	4.1	4.72
Diet	91.1	5.31	2.3	2.52
Over 3 Laboratories (3, 5, and 7)				
10% Protein diets (5):				
Nitrogen	90.2	0.74	0.86	0.95
Diet	89.9	0.24	0.49	0.54
6% Protein diets (3):				
Nitrogen	85.1	3.01	1.74	2.04
Diet	90.0	0.36	0.60	0.67

^aCalculated on a moisture-free basis.^bCoefficient of variation = SD/mean × 100. SD = $\sqrt{\text{variance}}$.

The data also clearly indicated differences in digestibility between protein sources, and therefore the method is useful and valuable for estimating apparent nitrogen digestibility of a food protein source. It will also be useful for correlating in vitro and in vivo digestibility data, and in developing an in vitro method for estimating apparent nitrogen digestibility.

REFERENCES

- Oser, B. L. (1959) in *Protein and Amino Acid Nutrition*, A. A. Albanese (Ed.), Academic Press, New York, NY, p. 281
- Atwater, W. O., & Bryant, A. P. (1900) *The Availability and Fuel Value of Food Materials*, Conn. Storrs Agric. Exp. Stn. 12th Ann. Rep. (1899), pp. 73-110
- Merrill, A. L., & Watt, B. K. (1955) in "Energy Value of Foods—Basis and Derivation," *U.S. Dep. Agric. Handb. No. 74*, U.S. Dept of Agriculture, Washington, DC
- Watt, B. K., & Merrill, A. L. (1963) in "Composition of Foods," *U.S. Dep. Agric. Handb. No. 8*, U.S. Dept of Agriculture, Washington, DC, pp. 159-161
- Hopkins, D. T. (1981) "Effects of Variation in Protein Digestibility," in *Protein Quality in Humans: Assessment and In Vitro Estimation*, C. E. Bodwell, J. S. Adkins, & D. T. Hopkins, (Eds.), AVI Publishing Co., Inc., Westport, CT, pp. 169-193
- "Nutritional Evaluation of Protein Foods" (1980) P. L. Pellet & V. R. Young (Eds.), WHTR-3/UNUP-129, The United Nations University, Shibuya-ku, Tokyo 150, Japan, pp. 26-32, 51
- Bodwell, C. E. (1981) "Use of Amino Acid Data to Predict Protein Nutritive Value for Adults," in *Protein Quality in Humans: Assessment and In Vitro Estimation*, C. E. Bodwell, J. S. Adkins, & D. T. Hopkins (Eds.), AVI Publishing Co., Inc., Westport, CT, pp. 340-373
- Ritchey, S. J., & Taper, L. J. (1981) "Estimating Protein Digestibility for Humans from Rat Assays," in *Protein Quality in Humans: Assessment and In Vitro Estimation*, C. E. Bodwell, J. S. Adkins, & D. T. Hopkins (Eds.), AVI Publishing Co., Inc., Westport, CT, pp. 306-315
- Harper, A. E. (1981) "Task Force II Report," in *Protein Quality in Humans: Assessment and In Vitro Estimation*, C. E. Bodwell, J. S. Adkins, & D. T. Hopkins (Eds.), The AVI Publishing Co., Inc., Westport, CT, pp. 417-420
- Hackler, L. R. (1978) *Food Technol.* 32(12), 62-64
- Hackler, L. R., et al. (1984) *J. Assoc. Off. Anal. Chem.* 67(1), 66-77
- Happich, M. L., et al. (1984) *J. Assoc. Off. Anal. Chem.* 67, 621-622
- Mitchell, H. H. (1924) *J. Biol. Chem.* 58, 873-903
- National Academy of Sciences (1963) *Evaluation of Protein Quality*, Publication 1100, p. 63
- Harper, A. E. (1974) in *Improvement of Protein Nutrition*, National Academy of Sciences, Washington, DC, pp. 1-7
- Bressani, R. (1977) in *Evaluation of Proteins for Humans*, C. E. Bodwell (Ed.), The AVI Publishing Co., Inc., Westport, CT, pp. 81-118
- Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA, secs 2.057 and 24.003